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13. ABSTRACT ***(Maximum 200 Words)***

Previous studies conducted at the AERL EPA laboratories and in our labs have shown that TNT is rapidly transformed by a number of common aquatic plants. Currently, studies are being completed to assess the final fate of transformation products in whole plant systems. These studies demonstrate the assimilation of TNT-carbon into the plant structure, at rates less than initial transformation.

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TRANSFORMATION OF TNT BY AQUATIC PLANTS

J.B. Hughes, Ph.D.
Assistant Professor
Department of Environmental Science and Engineering
George R. Brown School of Engineering
Rice University

Joseph B. Hughes

Dr. Joseph B. Hughes' field of research is applied microbiology and biological process engineering. Dr. Hughes joined the Rice faculty after completing his Ph.D. in 1992 at the University of Iowa, where his dissertation dealt with anaerobic biodegradation — specifically, the transformation of chlorinated aliphatics by methanogenic cultures. The focus of much of Dr. Hughes'' research to date has been the use of biological processes to remediate hazardous wastes. As a member of the Department of Environmental Science and Engineering at Rice University, Dr. Hughes teaches courses in Water and Wastewater Treatment, Biological Process Engineering, and Environmental Microbiology.

Dr. Hughes' work in the area of chlorinated solvent biodegradation has focused on the effect of mixtures of chlorinated aliphatics on the biotransformation rates of individual components of the mixture, concentration effects on chlorinated aliphatic transformation kinetics, and the role of electron donors on transformation processes. Such studies provide information that will be needed to evaluate the feasibility of biological processes in remediating contaminated media. This work on chlorinated aliphatics has been published in the ASCE *Journal of Environmental Engineering, Water Science and Technology,* and <u>In situ and On-site Bioreclamation</u>.

In addition to continuing research in this area, Dr. Hughes is studying various approaches to facilitating bioremediation of PAH-contaminated sediments. He recently published a paper in *Environmental Toxicology and Chemistry* on the use of commercial surfactants to enhance the bioavailability of these compounds. He is currently working on the engineering of slurry reactors to optimize their performance in degrading PAH-contaminated sediments with indigenous organisms.

A current emphasis of Dr. Hughes' ongoing research addresses munitions biodegradation in bacterial and plant systems. His investigations into trinitrotoluene metabolism, transformation pathways and end-products are in collaboration with researchers at other major universities and national laboratories.

3:30 **Transformation of TNT by Aquatic Plants**

J. B. Hughes, Ph.D., Assistant Professor, Department of Environmental Science and Engineering, George R. Brown School of Engineering, **Rice University**

Previous studies conducted at the AERL EPA laboratories and in our labs have shown that TNT is rapidly transformed by a number of common aquatic plants. Currently studies are being completed to assess the final fate of transformation products in whole plant systems. These studies demonstrate the assimilation of TNT-carbon into the plant structure, at rates less than initial transformation.

4:00 **Phytoremediation of Explosives in Aquatic-Plant Systems: Process Kinetics, Design and Performance**

F. Michael Saunders, Ph.D., Professor of Environmental Engineering, **Georgia Institute of Technology**

Explosives in contaminated groundwaters and soils have been shown to be persistent in the natural environment at ammunition plants and assembly facilities. Aquatic plants have been investigated as to their ability to transform trinitrotoluene (TNT) and other explosives. Explosive reduction through phytochemical amination, conjugation and incorporation into plant tissue have been successfully demonstrated. Kinetic relationships for plant transformation of TNT and byproducts by plants (e.g. *Elodea densa* and numerous submergent and emergent plants) will be presented. These relationships have been investigated in laboratory and field systems and, design and performance data will be presented.

4:30 **Phytoremediation of Explosives-Contaminated Groundwater in Constructed Wetlands**

Darlene F. Bader, Program Manager, Environmental Technology Division,
U.S. Army Environmental Center

The U.S. Army Environmental Center is leading a program to use constructed wetlands to remediate explosives-contaminated groundwater. A plant enzyme system has successfully degraded the explosives TNT and RDX to environmentally acceptable products during laboratory studies. Treatability studies are consistent with laboratory findings. An overview of the Army program and results will be presented.

5:00 **Roundtable Discussion**

5:30 *Close of Day One* Lounge tonite - informal

Thursday, May 9, 1996

8:25 *Poster/Exhibit Viewing and Coffee**

8:55 **Chairperson's Opening Remarks**

Alan J. M. Baker, Ph.D., Reader in Environmental Science & Environmental Consultant, Department of Animal and Plant Sciences, **University of Sheffield, UK**

Session III Inorganic Contaminants

9:00 **Making Phytoremediation Work**

Ilya Raskin, Ph.D., Professor, Center for Agricultural Molecular Biology, **Rutgers University**

Toxic metal pollution of waters and soils is a major environmental problem facing the modern world. The use of specially selected and engineered metal-accumulating plants for environmental clean-up is an emerging technology which has been called phytoremediation. Two subsets of this technology are being developed: Phytoextraction: the use of metal-accumulating plants, which can transport and concentrate metals from the soil into the harvestable parts of roots and above-ground shoots, and Rhizofiltration: the use of plant roots to absorb, concentrate and precipitate toxic metals from polluted effluents. Recent advances in phyto-remediation technology as well as its potential problems will be discussed.

9:30 **The Use of Different Plants ... Concentrations in California Soils**

Gary Banuelos, Ph.D., Plant/Soil Scientist,
USDA-ARS, Water Management Research Laboratory

Natural weathering of Cretaceous shale, in conjunction with irrigation practices on Se-laden soils, redistributed Se salts to shallow aquifers, streams and/or wetland areas. Improving irrigation management and growing crops that take up large amounts of Se is a potential technique to remove soluble Se from these soils. Greenhouse and field results from the last 7 years have successfully demonstrated phytoremediation of Se-laden soils. Combining newly developed water management practices on selected plant species improves the technology of phytoremediation for inexpensively lowering high levels of soil Se in shallow contaminated soils.

10:00 *Poster/Exhibit Viewing and Refreshment Break**

10:45 **The Use of Phytoremediation in the Clean-Up of Selenium Polluted Soils and Waters**

Norman Terry, Ph.D., Professor of Environmental Plant Physiology, Department of Plant Biology, **University of California, Berkeley**

Toxic levels of selenium (Se) arise in agriculture from the irrigation of Se-rich soils and in industry from the processing of fossil fuels. The cleanup of Se from soils and industrial wastewaters can be achieved by using plants to remove Se and convert it to a non-toxic gas (phytovolatilization) which may be removed completely and safely from the local ecosystem. Progress in our research on the ecology, microbiology and molecular biology of phytovolatilization will be presented.

11:15 **Getting the Lead Out, or Leaving it in: The Art, Science (and a Little Politics) of the Phytoremediation of Lead Contaminated Soils**

Scott D. Cunningham, Ph.D., Principal Investigator,
DuPont - Central Research & Development

The talk will cover the basics of two types of low-input remediation: 1) an in-situ stabilization with soil amendments and plants, and 2) "phyto" (or plant-based) extraction of Pb. The latter topic will emphasize our efforts to select, grow and manipulate plants and soils to allow plants to accumulate greater than 1% Pb in their tissue as a remediation strategy. The plants are then harvested, and processed for Pb recovery or disposal.

11:45 **Roundtable Discussion**

12:15 *Lunch on your own*

1:40 **Chairperson's Remarks**

Alan J. M. Baker, Ph.D., Reader in Environmental Science & Environmental Consultant, Department of Animal and Plant Sciences, **University of Sheffield, UK**

1:45 **Metal-Accumulating Plants: The Biological Resource and its Commercial Exploitation in Soil Clean-Up Technology**

Alan J. M. Baker, Ph.D., Reader in Environmental Science & Environmental Consultant, Department of Animal and Plant Sciences, **University of Sheffield, UK**

All plants take up metals to varying degrees from the soils in which they are rooted. Plant responses to contaminated soils may involve accumulation of metals in biomass. Metal accumulation can range from a slight elevation relative to background concentrations to a significant percentage of the plant dry matter. Some plants endemic to metalliferous soils accumulate exceptionally high metal concentrations: these have been termed 'hyperaccumulators.' This presentation will review our present knowledge of metal hyperaccumulator plants in order to identify those with the greatest potentials for development as phytoremediator crops for metal-contaminated soils.

Transformation of TNT by Aquatic Plants

J. B. Hughes
Environmental Science and Engineering
George R. Brown School of Engineering
Rice University
Houston, Texas

Overall Project

In Situ Biochemical Remediation of TNT-Contaminated Soils:
Integrated Laboratory and Field Studies

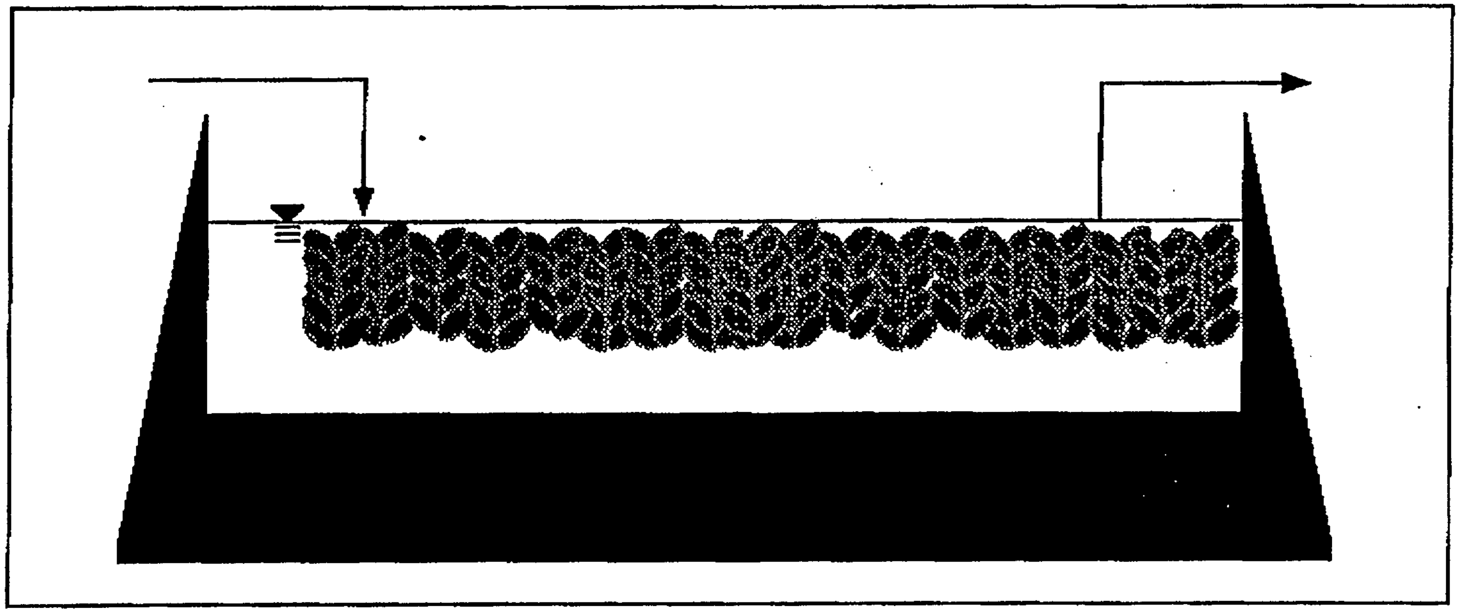
HSRC South & Southwest

LSU, Rice University, Georgia Tech

Investigators:

F.M. Saunders, J.B. Hughes, K. Ro
S.G. Pavlostathis, D.D. Adrian, R. Bhadra, D. Constant, D. Frost, M. Jacobson, J. Pierson, K. Qaisi, J. Shanks, L.J. Thibodeaux, C. Tiller, K.T. Valsaraj, X. Wang

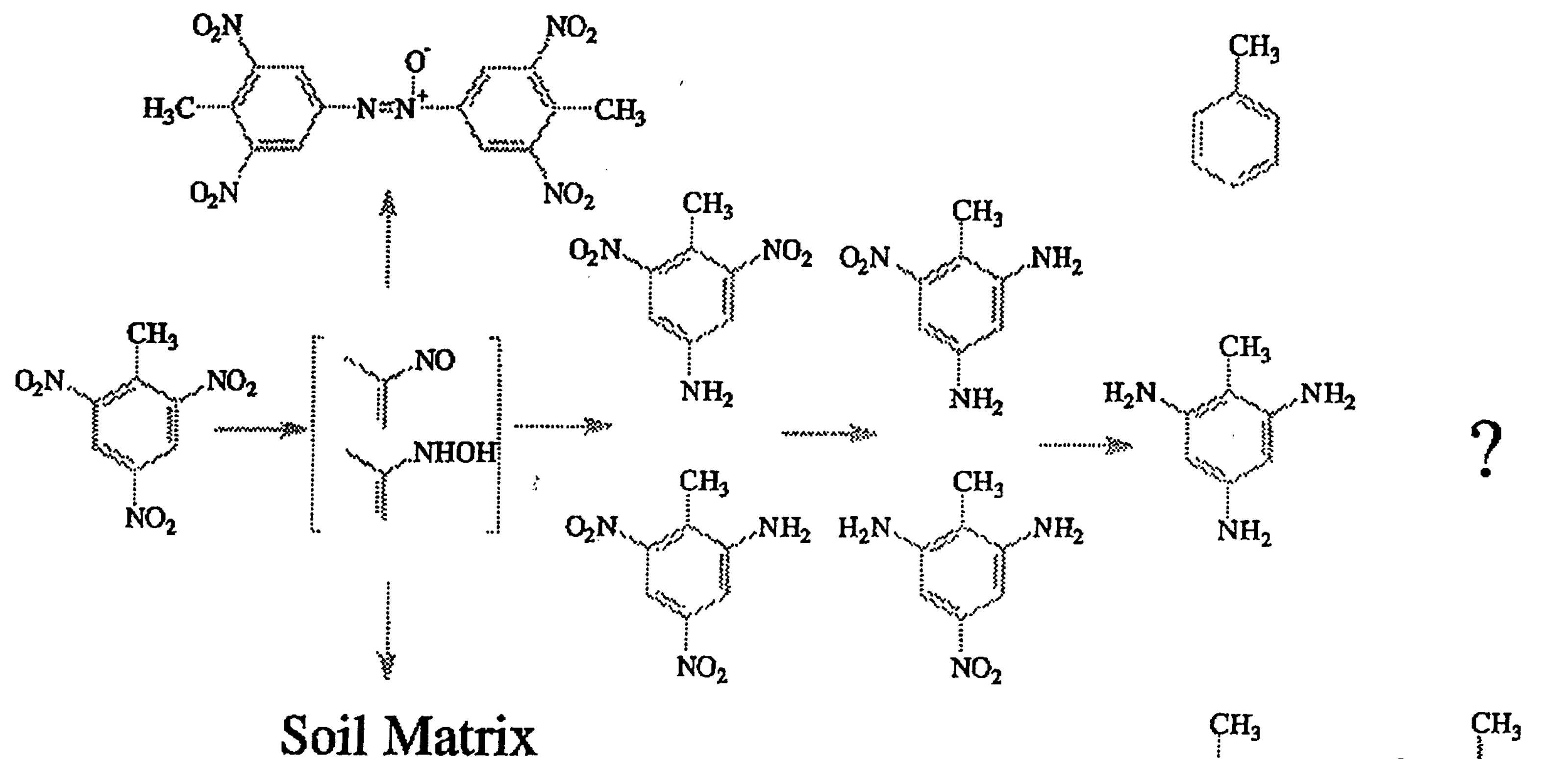
Lagoon Treatment System

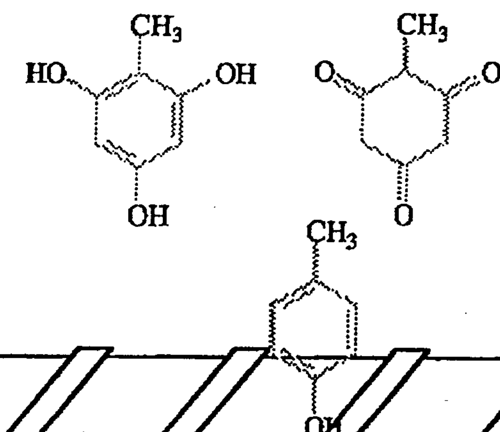


- *Hydraulic control*
- *Contaminated soil or ground water*
- *Plant species introduced and "maintained"*

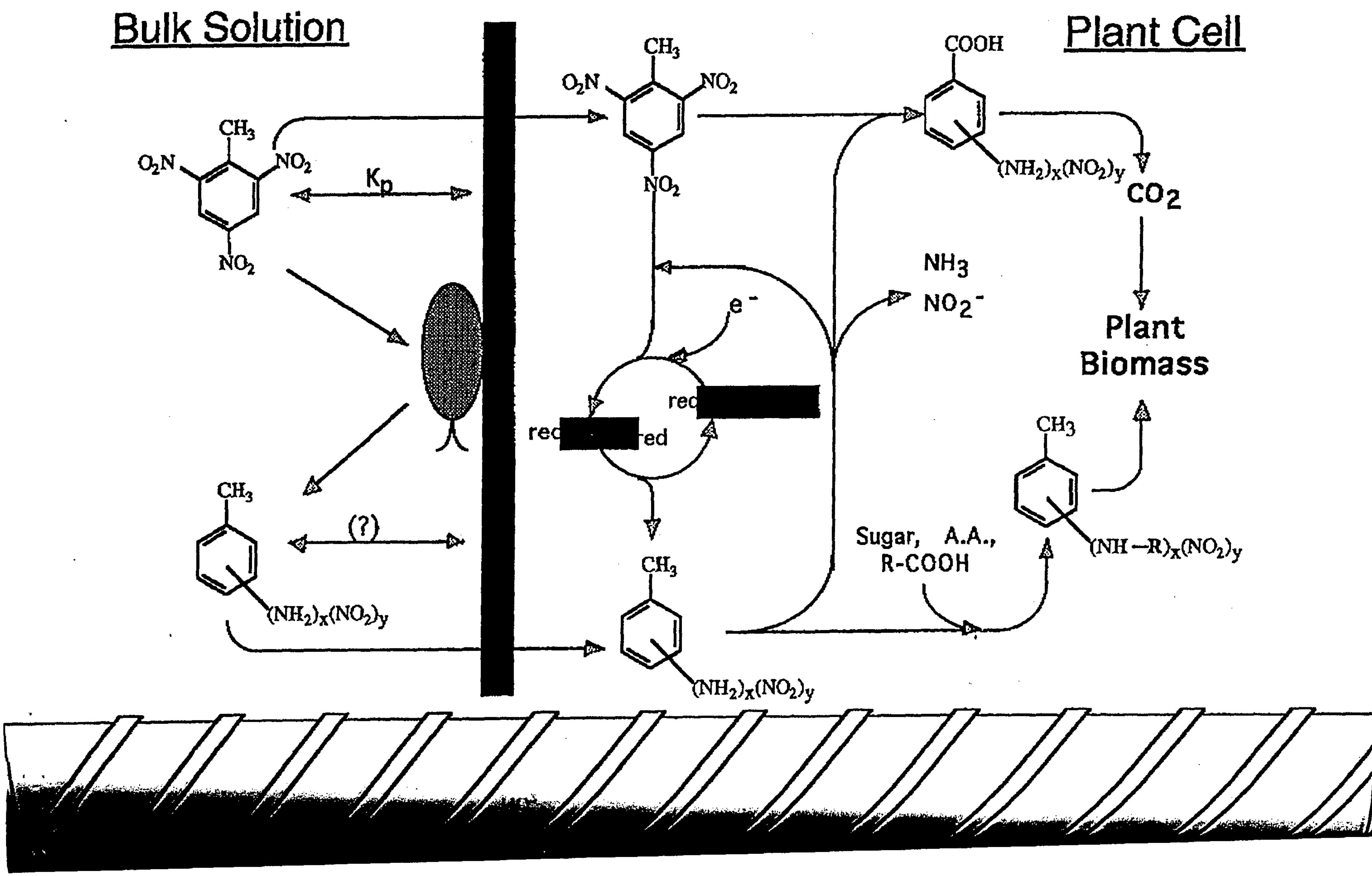
Research Needs

- Fate of TNT
- Process kinetics
- Stability
- Seasonal variability
- Competing reactions
- Limits of treatment





TNT transformation


Bulk Solution
Plant Cell
CH_3
O_2N
NO_2
NO_2
K_p
(?)
CH_3
$(NH_2)_x(NO_2)_y$
CH_3
O_2N
NO_2
NO_2
e^-
rec [redacted]
rec [redacted] ed
CH_3
$(NH_2)_x(NO_2)_y$
COOH
$(NH_2)_x(NO_2)_y$
CO_2
NH_3
NO_2^-
Plant
Biomass
Sugar, A.A.,
R-COOH
CH_3
$(NH-R)_x(NO_2)_y$

Plants Studied

Taxonomic Name	Common Name	Taxonomic Name	Common Name
Lemna sp.	Duckweed	*Rhizoclonium sp.*	Filamentous Algae
Myriophyllum aquaticum	**Parrot Feather**	*Eichornia crassipes*	Water Hyacinth
Myriophyllum spicatum	**Parrot Feather**	*Elodea sp.*	Waterweed Angiosp.
Ceratophyllum demersum	Hornwort	*Alternanthera philoxeroides*	Alligator Weed
Chara sp.	Stonewort	***Catharantheus roseus***	**Hairy Root**

Experimental Systems

- Batch experiments
- Initial TNT concentrations - 60 ppm to 100 ppm
- Amended with ^{14}C-TNT
- Monitor with HPLC, MECE, and ^{14}C
  - Aqueous phase
  - Plant surface
  - Plant extract (MeOH/acetonitrile)
  - mineralization

Growth Conditions

- Native
- Hydroponic
- Axenic

Screening Studies

Plant	TNT Activity	Toxicity
Lemna sp.	+	yes
Myriophyllum aquaticum	+ (warm)	not apparent
Myriophyllum spicatum	+ (cold)	not apparent
Eichornia crassipes	+	not apparent
Elodea sp.	+	not apparent
Alternanthera philoxeroides	+	not apparent

Fate of TNT

- TNT rapidly disappears from media
- Reduction products observed
- Mineralization is <u>not</u> observed
- A significant fractionassociates with plant
- May or may not be extractable
- Soluble products are observed

Summary

- Many plants demonstrate capability
- End products not “typical”
- Products associate, in part, with biomass
- Activity appears to be plant based
- Rates amenable to engineered system

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- Ron Spanggord